Proposal
5-4727A
for

ESR STUDIES OF BIOCHEMICAL EFFECTS OF ATMOSPHERIC POLLUTANTS

to

THE COUNCIL FOR TOBACCO RESEARCH, U.S.A.
633 Third Avenue
New York, New York

Attention: Dr. J. Morrison Brady
Associated Scientific Director

August 3, 1966

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In our proposal #5-4727, I indicated that I had observed an electron paramagnetic resonance signal from an homogenate of a freshly excised rabbit lung into which had been smoked six popular brand cigarettes. A short pre-print of a publication, Appendix A, which outlines these results is included with this proposal supplement.

In a telephone conversation I was asked if I could give some additional information to strengthen my grant application. I believe the following discussion will supply the necessary information.

As indicated in Appendix A, I believe the observed electron paramagnetic resonance pattern is produced by the interaction of free radicals and/or reactive diamagnetic molecules with a metal complex present in the rabbit lungs. To be more specific I believe the bulk of the observed signal is due to the reaction of these free radicals and/or reactive molecules with the haemoglobin molecules present in the red blood cells. Such an interaction is represented in Figure 1. In the haemoglobin molecule the iron is present as a ferric ionic complex i.e. Fe^{3+}. The paramagnetic resonance spectra of Fe^{3+} ionic complexes have been investigated by many workers. In particular however, detailed electron spin resonance studies have been performed on four derivatives of the haemoglobin molecule, in order to discover the detailed form of bonding between the central iron atom and the particular group on the sixth coordination point, the point at which oxygen attachment occurs.

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The tentative explanation of the reported ESR signal from smoked rabbit lung is the attachment of an active molecule (s) from tobacco smoke to the selfsame sixth coordination point. On the basis of this hypothesis several

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extremely important questions arise:

1. Is this the transport mechanism to the liver which leads to the formation of acetonitrile by the liver?

2. Why does the lung membrane pass these molecules so readily?

3. What molecules or radicals are thus passed and picked up and are these identical to those in smoke?

4. Is there a connection between these modified haemoglobin molecules and the observed vessel constriction in smokers?

Since none of these questions lie within the scope of our present NCI program (primarily because of the unexpected nature of this discovery), TRC support is essential to allow the investigation of any or all of these. Before we attempt an exploration of the more complex biological questions raised above, there are several rather direct questions which must first be dealt with; for example:

1. What types of molecules and/or radicals will react to form haemoglobin complexes such as we have observed?

2. What is the stability of these complexes at physiological conditions?

3. Are such complexes formed by other atmospheric contaminents; i. e., smog, automobile exhaust fumes, etc. ?

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THEORETICAL CONSIDERATIONS

The structure of the central portion of the haemoglobin molecule is represented in Fig. 1 where the haem-porphyrin plane is shown perpendicular to the axis joining the iron atom to the globin molecule and the ligand at the sixth coordination point. The main feature of the haemoglobin molecule is the relative ease with which the ligand of the sixth coordination point can be changed which makes it feasible to study a whole series of derivatives formed by substituting different ligands into this position.

Single crystal studies have been made on the acid methaemoglobin, acid metmyoglobin, metmyoglobin fluoride and metmyoglobin azide. The extreme g factors for each derivative are listed in Table I.

TABLE I

Derivative	Group attached	$g_{\parallel}$	$g_{\perp}$	μ in Bohr magnetons
Acid methaemoglobin	H_2O	2.0	6.0	5.84
Acid metmyoglobin	H_2O	2.0	6.0	5.85
Metmyoglobin fluoride	F	2.0	6.0	5.92
Metmyoglobin azide	N_3	2.8	1.70	2.84

The parallel suffix refers to directions parallel to the axis through the iron atom normal to the haem plane and the perpendicular suffix to directions at right angles to this axis (i.e., in the haem plane). It can be seen from Table I that the four derivatives so far studied are divided into two classes by both

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the resonance and susceptibility measurements. As a result of the susceptibility data these two groups were classified as "essentially ionic" and "essentially covalent" corresponding to the cases of a spin quantum number S = 5/2 or 1/2. Thus, if ionic binding is assumed, the central positively charged ferric ion will be left with five 3d electrons. These will line up with their spins parallel according to Hund's rule and so half fill each of the five vacant 3d orbitals. A total electronic spin moment of 5/2 is thus formed which will itself be quantized in any applied field. On the other hand, if covalent bonding takes place six pairs of electrons are required to form with the surrounding ligands. These electrons donated by the surrounding atoms will occupy certain orbitals of the ferric iron, and it can be shown that the most stable configuration for octahedral bonding is the one in which two 3d orbitals and the one 4s orbital and three 4p orbitals are employed. The five non bonding 3d electrons of the ferric iron are therfore, left with only three 3d orbitals to occupy and hence four are forced to pair leaving one unpaired and a resultant S = 1/2.

If this simple interpretation were correct the resonance spectrum obtained from the essentially ionic compounds would be expected to have the same general features as that of the other ionic ferric salts. Thus, the total spin and associated magnetic moment corresponding to the configuration with S = 5/2 would take up six different orientations varying from +5/2 to -5/2. Transitions can then be induced between each of these levels according to the selection rule $\Delta S = \pm 1$ and thus five electronic absorption lines would be expected centered on a g factor of 2.0. However, the work on single crystals of

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acid methaemoglobin, metmyoglobin and metmyoglobin fluoride showed that the g factor was in fact, anisotropic varying from a $g_{//}$ of 2.0 to a $g_{\perp}$ of 6.0, and that there was only one electronic transition observable. A theoretical explanation of the observed anisotropic values has been provided by assuming that very strong asymmetrical crystalline fields are active on the iron atoms so that the energy difference between the six different orientations of the total spin vector of 5/2 are much larger than the microwave quanta.

This highly anisotropic g factor of the "essentially ionic" haemoglobin derivatives makes the detection of the paramagnetic resonance signal of amorphous samples difficult, since the resonance line extends from approximately 1300 gauss to 3250 gauss. This of course, accounts for the each of any apparent signal from the sample of untreated rabbit lungs.

In contrast to the large variation from g = 2 to g = 6 observed for the ionic derivatives the azide derivative which is "essentially covalent" has a g factor spread across the free spin value, indicating an S of 1/2 with considerable orbital interactions. The signal we observe from rabbit lungs which have been treated with tobacco smoke is also located at approximately the free spin value. Consequently, we have tentatively attributed at least part of our observed signal to "essentially covalent" derivatives of haemoglobin being formed by reaction of the normal haemoglobin with constituents of the tobacco smoke.

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So far the above discussion o the nature of the resonance is hypothesis based upon what is known about iron in other complexes and has

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to be verified experimentally. However, assuming the hypothesis is correct, such a complex provides a vehicle for carrying the chemicals present in cigarette smoke to the liver for the production of for example, acetonitrile.

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PROPOSED PROGRAM

1. To establish that the origin of our observed electron spin resonance signal is in fact as we have outlined.

2. To determine the types of molecules and free radicals that will react with haemoglobin to produce the type of complex we have observed from the reaction of tobacco smoke with haemoglobin. In this study we will restrict ourselves to compounds which are normally found in tobacco smoke, smog, and automobile exhaust. Since all three systems contain many constituents in common, at this stage we will restrict ourselves to the investigation of representative examples of these common species.

3. Having established the necessary requirements for the production of such a complex, to investigate the stability of the complex, i.e., is the complex once formed stable or is the decomposition of the complex to reform undamaged haemoglobin a probable reaction?

4. To determine what effect the formation of such a complex has on the general reactivity of the haemoglobin molecule. Since the bonding of the iron atom to both the porphyrin ring and the globin molecule changes upon formation of such a complex this is accompanied by a change in electron distribution throughout the whole molecule. Such a change in electron distribution will necessarily cause changes in the chemical reactivities of different areas of this complex molecule.

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SUBMISSION OF REPORTS

A bi-annual report will be submitted to the Tobacco Research Council throughout the course of this program, with a final report summarizing the entire program. It is anticipated that much of this work will be appropriate for publication in the open Scientific Literature, but prior to publication the Sponsor requested to review the manuscript and give his consent to the publication.

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APPENDIX A.

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